

TITLE OF THE INVENTION

Chitosan-xanthan based polyionic hydrogels for stabilisation and controlled release of vitamins

5 FIELD OF THE INVENTION

The present invention relates to chitosan/xanthan based hydrogels. More specifically, the present invention deals with chitosan/xanthan based hydrogels used in dermatology and as food additives, where such hydrogels are used as carrier for active compounds such as vitamins, amino-acids, nucleic acids, polypeptides, etc.

10 BACKGROUND OF THE INVENTION

The production of chitosan/xanthan hydrogels is known. US patents 5,620,706 and 5,648,252 describe such hydrogels as inert supports for enzyme immobilisation or for the controlled release of specific antibiotics or anticancer agents. However, the application of such hydrogels as supports, stabilisation and subsequent controlled release of vitamins, amino-acids, nucleic acids and polypeptides has not been proposed yet.

20 One of the key aspects in the preparation of food additives and dermatological preparations is the preservation of active ingredients prone to degradation, such as vitamins, amino-acids, nucleic acids and polypeptides. Exposure of such ingredients to heat or light accelerates their degradation.

25 Given the importance of the active ingredients mentioned above, several excipients, such as tablets, capsules, gellules, gels, lotions, ointments, emulsions and simple solutions, have been developed with a view to protect the active ingredients against degradation or simply to render them

09301670.04389
663240.02910E60

hydrophobic. However, several drawbacks of these synthetic devices have been identified. In particular their irritation potential and toxicity were put forward.

5 Due to the inherent limitations of conventional excipients, the use of synthetic polycations was recently proposed. As an example, European patent application # 504 066 A1, 1992, to L'Oréal (France) proposes cosmetic compositions comprising a dispersion of active solid particles coated with a cationic polymer. The aim of the polymeric coating being to
10 increase the stability of the overall composition in addition to preventing degradation of the active ingredient.

Yet an important objective remains unfulfilled. Indeed, there remains an important need to develop new excipients capable of being used in food and dermatological preparations to stabilise thermo- or photo sensitive active
15 ingredients, such as vitamins, nucleic acids, amino-acids and polypeptides.

OBJECTS OF THE INVENTION

An object of the present invention is therefore to provide a hydrogel preparation for use as a delivery device for food or dermatological
20 preparations with a concurrent objects of providing stabilization of thermo- and photo sensitive active ingredients and avoiding any irritation or toxicity potentials. A further object of the present invention is to disclose a method of dispersion of the active ingredient within the hydrogel matrix.

25

SUMMARY OF THE INVENTION

Generally, in accordance with the present invention, there is provided a preparation which stabilises thermo- and photo sensitive bioactive molecules. The preparation comprises a hydrogel made of a complex of

chitosan and xanthan. Within the hydrogel there is lodged at least one thermo- or photo sensitive substance chosen among the following: vitamins, amino-acids, nucleic acids and polypeptides. The hydrogel configuration and structure is prepared so as to release, in a controlled way, the thermo-
5 or photo sensitive ingredients either in a human or animal subjects.

The present invention also discloses a method of making these hydrogels. The present invention also teaches the use of these hydrogels in dermatology or as a food additive vehicle.

10

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non restrictive description of preferred embodiments thereof, given by way of example only with reference to the accompanying drawings.

15

Those specialised in the area covered by this invention will certainly be able to apply modifications or adaptations to the details described in the preferred embodiment while being constrained within the framework of the current invention.

20

BRIEF DESCRIPTION OF THE DRAWINGS

In the appended drawings:

- Figure 1 is a complex of chitosan and xanthan;
Figure 2 is a diagram of the lab apparatus for kinetic studies;
25 Figure 3 is an amount of Vitamin C released as a function of time/
Sample coded VS2L. The hydrogel CHITOXAN™ –
Vitamin C is prepared with CHITOXAN™ having a
swelling index (α) of 1800%;

568240-029F0E50

Figure 4 is the rate of release of Vitamin C as a function of time. Sample coded VS2L;

Figure 5 is the amount of Vitamin C released as a function of time/ Sample coded VS2R. The hydrogel CHITOXAN™ – Vitamin C is prepared with CHITOXAN™ having a swelling index (α) of 2200%;

Figure 6 is the rate of release of Vitamin C as a function of time. Sample coded VS2R ; and

Figure 7 is the variation of the % of Vitamin C released as a function of time. Sample coded VS2M. The hydrogel CHITOXAN™ – Vitamin C is prepared with CHITOXAN™ having a swelling index (α) of 3500%.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The detailed description reveals compositions for food and dermatological applications comprising one or several active ingredients that are either prone to thermo- or photo degradation. These compositions are based on a polyionic hydrogels obtained by a chitosan/ xanthan complexing which incorporates therein the active ingredients thereby protecting them from thermo- or photo induced degradation while controlling their release thus enhancing their activity and the duration of this activity.

Also described are two methods of incorporation of the active ingredients in the hydrogel depending on the nature of the active ingredients. In a first method, liposoluble active ingredients are introduced in the hydrogel during the making of the hydrogel. In a second method, hydrosoluble active ingredients are introduced by diffusion in the already made hydrogel. Both methods can be used in sequence.

The present invention thus reveals a novel and surprising way of introducing various vitamins and other ingredients such as amino-acids, nucleic acids and polypeptides in hydrogels and of controlling the release of those ingredients in various drug delivery vehicles such as: capsules or gelcaps for
5 oral ingestion, rectal suppositories, creams and ointments, gels, solutions and cutaneous patches.

The present invention further reveals a method of introducing in the same hydrogel liposoluble and hydrosoluble active ingredients.
10

Finally, the present invention further discloses methods of making food additives and dermatological creams incorporating the hydrogel.

It is to be noted that the terms "dermatologic" and "dermatological" are used in their widest sense thus covering both the dermatological and cosmetic applications. Furthermore, these terms are meant to cover direct skin treatments or treatment through nails or hair.
15

The term "food additive" is also to be understood in its widest sense including thus all food preparations where the additive has a nutritional or therapeutic function as well as simple mechanical functions such as that of a texturizing agent, filler or viscosity control agent.
20

The preparations covered by the present invention focus mainly on humans although they may be used in the veterinary field.
25

On figure 1, it is shown how the hydrogel of the present invention is an ionic complex between chitosan, a cationic natural polymer, and xanthan, an anionic natural polymer. A method of making of the hydrogel has been

662240-02910650

described in US patent 5,720,206 granted to the same assignee as in the present application and is incorporated herein by reference. As shown in Figure 1, xanthan and chitosan form a complex, i.e. a network of ionic linkages between these two polymer molecules. The complex is a highly hydrophilic hydrogel.

(a) Inclusion of liposoluble vitamins in the chitosan-xanthan hydrogel

Vitamin A, also called Retinol, is a molecule extremely sensitive to light and oxygen. As a consequence, this vitamin may not be used in a cream unless it is being stabilised to counteract the negative effects of light and oxygen (from ambient air). The method of the present invention consists in stabilising Vitamin A in a hydrogel made of xanthan and chitosan complex. The xanthan and chitosan complex being described in US patent 5,620,706.

Example 1: Inclusion of Vitamin A when making the hydrogel

A solution (100 ml) of Vitamin A (10-20 w/v%) in ethanol is first prepared. This solution is added, under intense agitation, to 500 ml of a xanthan solution, 0.65w/v%. The final solution has a Vitamin A concentration between 1.66 and 3.33 w/v% . The solution is kept at 3°C. It is subsequently sprayed, via a nozzle, into 800 ml of a 0.65w/v% chitosan solution. The complexation reaction is conducted during 30 minutes. The gel formed is then filtered and rinsed with water to a pH of 6.8. In order to increase the hydrogel stability, a final washing with a sodium bicarbonate solution, 1 w/v%, brings the gel to a pH of 7.5. The gel is then frozen and freeze-dried. All these operations, including the freeze drying, are best conducted in the absence of light and oxygen.

Example 2: Inclusion of Vitamin A by diffusion

Using a previously prepared xanthan-chitosan hydrogel with a swelling index (α) of at least 2000%, it is possible to introduce the Vitamin A by diffusion. The swelling index (α) is defined as follows:

$$\alpha = 100\% \times \frac{\text{mass of swelled hydrogel in equilibrium} - \text{mass of dried hydrogel}}{\text{mass of dried hydrogel}}$$

Under these conditions, the time for the experiment is decreased thereby preventing the molecule's degradation. The method involves dissolving 0.07 g of Vitamin A in 1 ml of ethanol (96%) and adding 1.5 g of the freeze dried xanthan-chitosan complex having $\alpha = 2500\%$. Slight agitation allows the obtention of a homogeneous paste. 2 ml of ethanol, and 200 μ l of water are then added under slight agitation and the mixture is kept at 4°C for 24 hours in absence of light. The alcohol is then evaporated, at 4°C. The final product can be freeze dried and has 46 mg of Vitamin A per g of freeze dried xanthan-chitosan complex.

Example 3: Inclusion of Vitamin E

The method (a) developed for Vitamin A is also applied for the inclusion of Vitamin E. The concentration of Vitamin E in the freeze dried hydrogel can reach 20 wt% .

Example 4: Inclusion of Vitamin K

The method (a) developed for Vitamin A is also applied for the inclusion of Vitamin K. The concentration of Vitamin K in the freeze dried hydrogel can reach 20 wt% .

(b) Inclusion of hydrosoluble vitamins in the chitosan-xanthan hydrogel

For bioactive hydrosoluble ingredients, it is preferable to use the diffusion method in the freeze dried hydrogel to avoid loss of ingredients that would

09301670-042899

5 Example 5: Inclusion of Vitamin C

Step 1: Preparation of the xanthan-chitosan complex (CHITOXAN™), i.e. the polyonic hydrogel;

Step 1- Preparation of the xanthan-chitosan complex

Step 2 - Incorporation of Vitamin C

2a. Stabilisation with amino-acids

To 10 ml of water are added 1 g of Vitamin C, 0.06 g of L-cysteine, 0.02 g of L-cystine and 0.02 g of L-methionine. To this solution, 1 g of freeze-dried CHITOXAN™ made of particles having diameters comprised between 250 and 500 μm is added. It is not necessary that the mixture have any excess liquid. Pure water may be added to complete the hydration for a period of 2 hours. All the operations preferably require the absence of light. The

mixture is then frozen, freeze-dried and milled to provide a final product made of particles having diameters comprised between 50 and 125 μm .

The Vitamin C thus incorporated in the freeze-dried hydrogel and hydrated shows a good stability, without coloration after 2 weeks at 45°C (wet hydrogel) and after 20 weeks at 45°C (dried hydrogel). It is also possible to use as "stabilisers" either tartaric acid at 0.1 wt%, metaphosphoric acid at 0.03 wt% or citric acid at 0.1 wt%. The percentage is expressed with respect to CHITOXAN™.

2b. Stabilisation with tripeptides

The previous method (2a) is used replacing the three amino-acids with a tripeptide having sulfur-containing functionalities. To 10 ml of water, g of Vitamin C and 0.002 g of glutathione are added. After 5 minutes of agitation, 1 g of CHITOXAN™ is added. The mixture is kept in slight agitation until a homogeneous paste is obtained. The mixture is left to stand for 2 hours to reach the equilibrium hydration. The paste is then frozen and freeze-dried. All operations preferably require absence of light.

(c) **Extraction and determination of Vitamin C included in CHITOXAN™**

Extraction

The solvent used is an aqueous mixture of 3% (w/v) metaphosphoric acid and 8% (v/v) acetic acid.

The method consists of introducing 20 to 30 mg of freeze-dried CHITOXAN™ loaded with Vitamin C in a 50 ml centrifuge tube together with 40 ml of the extraction solvent. The mixture is magnetically stirred for 60 minutes. The suspension formed is centrifuged (4000 rpm) and the supernatant is analysed. All operations are done in absence of light.

09301670-042999

Analytical determination of Vitamin C

This determination requires the establishment of a calibration curve at a maximum absorption wavelength using a UV-vis spectrophotometer. A standard is prepared with the same solution (and solvent) initially used to
5 introduce the Vitamin C within the CHITOXAN™. Experimentation has shown an absorption maximum at 243 nm for Vitamin C at 98% purity obtained from ALDRICH.

The calibration curve is constructed at 243 nm as follows. A solution of 1.3
10 mg/ml of Vitamin C in the solvent is prepared in a graduated cylinder. The solution is prepared just before analysis. By successive dilutions, the absorption versus concentration calibration curve (mg/ml) is thus measured.

The concentration of Vitamin C in the supernatant obtained via extraction
15 is determined at 243 nm using the calibration curve. The concentration of Vitamin C in the sample prepared by method 2a is 49.6%.

(d) Inclusion of the CHITOSAN™ – Vit. C preparation in a cream

1 g of CHITOXAN™ – Vit. C preparation is hydrated with water until a
20 creamy paste is obtained. Weighing the paste permits to calculate the amount of Vitamin C present. Subsequently, the paste is mixed, under strong agitation, with a base cream in order to achieve a final concentration in Vitamin C preferably comprised between 5 and 25 wt%.

(e) Determination of the Vitamin C stability in the cream base

10 g of the preparation obtained by inclusion of CHITOXAN™ – Vit. C in a cream base is introduced in a glass tube protected from exposure to light. The tube is heated at 45°C. In order to determine the rate of degradation of Vitamin C in the base cream, an aliquot is taken corresponding to a total

amount of 10 to 30 mg of Vitamin C. Such an aliquot is taken every day for the first four (4) days and, subsequently, every other day for a total of 30 days. Samples not immediately analysed were kept frozen at -4°C .

- 5 The aliquots were analysed with the method described earlier (always using the extraction solvent).

10 With samples VS2L, VS2R and VS2M, were prepared creams having concentrations of Vitamin C comprised between 5 and 25 wt%. Using the method previously described, Vitamin C inserted in CHITOXAN™ was found to be stable with no coloration of the cream appearing after 30 days at 45°C and the degradation of the Vitamin C was as little as 15 %. With a cream prepared with free Vitamin C (i.e. no hydrogel), an orange coloration appeared and a 98% degradation of Vitamin C occurred under the same conditions.

(f) Controlled release kinetics

20 Table 1 shows the types of CHITOXAN™ – Vit. C combinations studied. As illustrated in Figure 2, the controlled release kinetics is determined by introducing a precise quantity of the CHITOSAN™ – Vit. C preparation in the reactor.

Table 1: Types of CHITOXAN™ – Vit. C preparations studied

25	Preparations Codes CHITOXAN™ – Vit. C	Swelling index (α) of CHITOXAN™ %	Concentration of Vit. C in the CHITOXAN™ – Vit. C (wt%)
	VS2L	1800	50.3
	VS2R	2200	49.6
	VS2M	3500	51.0

The solvent, a mixture of 3% w/v methaphosphoric acid and 8% w/v acetic acid, flows into the central tube directly into the reactor where it comes into contact with the CHITOXAN™ – Vit. C preparation. The Vitamin C is gradually released from the preparation and solubilizes inside the solvent.

- 5 The latter leaves the reactor through small orifices ensuring constant circulation of the solvent and good contact with the preparation.

Figure 3 shows that the Vitamin C released from sample VS2L follows a linear increase as a function of time with two distinct slopes, depicted in Figure 4.

Figure 4 shows that with sample VS2L, the Vitamin C diffuses at a constant speed of 0.36 mg/min during the first period while it decreases by half for the 100 following minutes.

Sample VS2R also shows in Figure 5 the two distinct linear increase profiles with two distinct slopes depicted in Figure 6. The first slope is faster than that of preparation VS2L indicating a more rapid liberation. The second slope, at 0.02 mg/min, releases Vitamin C at a controlled but slow rate.

Figure 7 shows the liberation of Vitamin C for preparation VS2M. The release of 85% of the Vitamin C is achieved in the first 10 min.

The differences between the liberation profiles of the preparations are related to the swelling index. Sample VS2M, with a swelling index of 3500%, displayed a faster diffusion rate of Vitamin C than sample VS2R, $\alpha = 2200\%$, and the latter more rapid in turn than that of sample VS2L, $\alpha = 1800\%$. Thus the swelling index plays a key structural role in the release kinetics.

(g) Inclusion of CHITOXAN™ – Vit. C in a food mixture

Hydrogel CHITOXAN™ – Vit. C as well as other chitosan-xanthan hydrogels containing vitamins, nucleic acids, amino-acids and polypeptides can be used in hydrated food mixtures such as gels, sauces, syrups, etc. as well as
5 in dehydrated mixtures.

The physical characteristics of the chitosan-xanthan hydrogel will determine the structure and more or less viscous texture of the final products. The choice of a hydrogel as described in the present invention will permit to
10 adapt the hydrogel to diversified food applications.

Furthermore, the present invention also covers the production of tablets using CHITOXAN™ – Vit. C powder and other active ingredients as well when required. Thus, tablets made of freeze-dried powder of other chitosan-
15 xanthan hydrogels containing different vitamins, amino-acids, nucleic acids and polypeptides and their combinations can be prepared.

Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from
20 the spirit and nature of the subject invention as defined in the appended claims. It is obvious that the present application can accommodate numerous other variations within the framework of the invention as described.

668240-029TDE60